Early Wound Irrigation
Improves the
Ability to Remove Bacteria

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Background: Although most surgeons prefer to treat contaminated wounds as soon as possible, the effect of timing on the ability of irrigation to reduce the amount of bacteria in a wound is not fully known. We evaluated the effect of different delays in irrigation on bacterial removal in an animal model.

Methods: A complex musculoskeletal wound was created in the proximal part of the leg of goats. The wound was contaminated with *Pseudomonas aeruginosa* (lux) bacteria, genetically modified to emit photons, in order to allow for quantitative analysis of bacterial concentration with a photon-counting camera system. The contaminated wounds were closed, and wound irrigation was performed with 6 L of normal saline solution by means of pulsatile lavage after the assigned time-intervals of three, six, and twelve hours. Images were made before and after treatment. Relative luminescent units and clearance ratios were obtained and calculated for each wound.

Results: Earlier wound irrigation resulted in superior bacterial removal in our model. Irrigation resulted in a 70% ± 2%, 52% ± 3%, and 37% ± 4% reduction in bacterial counts from the pre-irrigation level at three, six, and twelve hours, respectively. The clearance ratios were significantly different at all time-points ($p < 0.004$).

Conclusions: Earlier irrigation in our contaminated wound model resulted in superior bacterial removal.

Clinical Relevance: While the actual bacterial counts necessary to establish a wound infection in humans is unknown, early irrigation of the contaminated wound is recommended for the prevention of infection.

The quantity of bacteria present in a contaminated open fracture wound correlates with the risk of development of wound and bone infection[1,2]. Therefore, one of the goals of initial irrigation and débridement of open fracture wounds is to decrease the bacterial load present in the wound as much as possible. The actual amount of bacteria present in a contaminated wound that will cause a clinical infection has not been determined. This bacteria quantity is dependent on the severity of the wound and the health status of the host. However, methods that reduce bacterial counts are considered to be advantageous in the treatment of contaminated wounds and open fractures.

The optimal timing of wound irrigation is controversial. While many studies from civilian trauma centers have shown no correlation between the timing of wound irrigation and clinical infection[3-9], one study of forty-seven grade-II and III open tibial fractures demonstrated a significantly higher infection rate in association with fractures that were treated more than five hours after trauma as compared with those that were treated before five hours[10]. A study of fractures that had been sustained during the Panama conflict demonstrated higher infection rates among soldiers who received initial irrigation after transportation to the United States as compared with those who received initial treatment in theater[11]. Unfortunately, all of those retrospective clinical studies are subject to potential selection bias. As a prospective, randomized trial would be difficult to perform, clinicians must rely on the few clinical studies and translational research performed with animal

Report Documentation Page		*Form Approved* *OMB No. 0704-0188*

1. REPORT DATE **01 AUG 2007**	2. REPORT TYPE **N/A**	3. DATES COVERED **-**
4. TITLE AND SUBTITLE **Early Wound Irrigation Improves the Ability to Remove Bacteria.**		5a. CONTRACT NUMBER
		5b. GRANT NUMBER
		5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S) **Owens B. D., Wenke J. C.,**		5d. PROJECT NUMBER
		5e. TASK NUMBER
		5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX 78234**		8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S)
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)
12. DISTRIBUTION/AVAILABILITY STATEMENT **Approved for public release, distribution unlimited**		
13. SUPPLEMENTARY NOTES		
14. ABSTRACT		
15. SUBJECT TERMS		

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT **SAR**	18. NUMBER OF PAGES **4**	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**			

models. We sought to evaluate the effect of different intervals between the time of injury inoculation and the time of treatment on bacterial removal with use of an animal model. We hypothesized that irrigation performed shortly after an injury would remove a greater proportion of inoculated bacteria than would irrigation performed after a longer delay.

Materials and Methods

All procedures were performed in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited laboratory after approval of the protocol had been obtained from the Institutional Animal Care and Use Committee. With use of appropriate general anesthesia, a reproducible complex musculoskeletal wound was created in the proximal part of the leg of goats as previously described[12]. Briefly, the wound involved exposure and thermal injury to the anterior compartment fascia; exposure, thermal injury, and crush injury to the tibialis anterior muscle; exposure and thermal injury to the tibial periosteum; and exposure and cortical damage by scoring of the tibial cortex.

The wound was inoculated with 1 mL of >10^8 CFU/mL *Pseudomonas aeruginosa* (lux), which was spread evenly over the wound surfaces with a sterile cotton-tipped applicator. This organism is genetically altered (by insertion of lux operon) to emit photons[13]. This concentration of inoculant is the highest that we can consistently obtain, and it demonstrated the greatest luminescence at the time of pre-irrigation imaging of the wound. The wound was left open for five minutes and then was stapled closed and bandaged.

After surgery, the goats recovered in their pens and were allowed activity *ad libitum*. At three, six, or twelve hours after surgery and inoculation, the animals were killed and were placed supine on the operating table. Ten animals were used for each time-point. Schanz pins were placed into the proximal part of the tibia, and the leg was mounted to the camera with an external fixation frame. The wound was re-opened, and a photon-counting camera (Charge Couple Device [CCD] Imaging System Model C2400; Hamamatsu Photonics, Hamamatsu-City, Japan) was utilized to capture the quantitative and spatial distribution of the bacteria in the wound. A black-and-white image was made, a photon count of the region was performed, and the data were quantified as relative luminescent units (RLUs).

Once the baseline luminescent data were collected, wound irrigation was performed with 6 L of saline solution with use of a pulsatile lavage device (InterPulse Irrigation System; Stryker Instruments, Kalamazoo, Michigan) operated at its highest setting. The pulsatile lavage system used a high-flow tip attachment (Model 210-14) with a maximum pressure of 19 psi and maximum flow rate of 1025 mL/min.

After treatment, images of the wounds were made to determine the post-irrigation values. Raw data were collected in the form of RLUs generated by the CCD camera and image processor. All data were saved in Microsoft Excel XP (Microsoft, Redmond, Washington). AQUACOSMOS imaging software (Hamamatsu Photonics) provided a count of RLUs

TABLE I Relative Luminescent Units (×10⁵) in Each Treatment Group*		
Treatment Group	Pre-Irrigation	Post-Irrigation
3 hour	4.63 ± 0.21	0.50 ± 0.08
6 hour	5.81 ± 1.11	2.87 ± 0.58
12 hour	5.26 ± 0.52	3.38 ± 0.46

*The values are given as the mean and the standard error of the mean.

for the entire field within view of the camera. RLU ratios were calculated for each time-group, with the pretreatment RLUs serving as the denominator. This calculation was necessary because of differences in pretreatment bacterial levels. RLU ratios for each time-group were analyzed with use of a mixed-model analysis of variance allowing for treatment, time, and the interactions among treatment and time as fixed effects. Preplanned orthogonal contrasts between the two treatments at each time-point were conducted. Analyses were carried out with use of the MIXED procedure in SAS (version 8.1, 1999; SAS Institute, Cary, North Carolina). All data are presented as the mean and the standard error of the mean. The level of significance was set at $p < 0.05$.

Results

Earlier wound irrigation resulted in superior bacterial removal in our model. The mean RLU values for the groups are listed in Table I. Irrigation at three hours resulted in a 70% ±
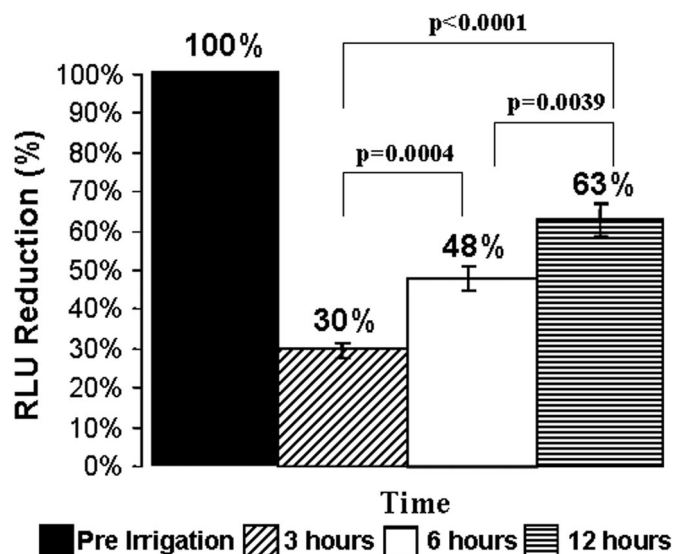

Fig. 1

Bar graph illustrating the percentage of relative luminescent units (RLUs) remaining after irrigation at three, six, and twelve hours after inoculation of wound. The pre-irrigation column (solid black bar) represents the baseline luminescence detected before irrigation.

2% reduction in bacterial counts from the pre-irrigation level. Only 52% ± 3% and 37% ± 4% of the bacteria was removed at six and twelve hours, respectively (Fig. 1). Significance was achieved for the difference in bacterial reduction between three and six hours (p = 0.0004), three and twelve hours (p < 0.0001), and six and twelve hours (p = 0.0039).

Discussion

In the present study, an established large-animal contaminated extremity wound model was used to evaluate the effect of irrigation timing on bacterial removal. Wound irrigation after a three-hour interval removed significantly more bacteria than did irrigation at six or twelve hours. The results in the six-hour group were also significantly superior to those in the twelve-hour group. These data support our hypothesis that earlier irrigation results in greater bacterial removal.

The time-intervals used in our study were chosen because they are clinically relevant. Three hours between injury and treatment represents the earliest scenario for definitive wound irrigation. The six-hour interval was chosen because this interval was approximately the average time to treatment in a recent large study of wound irrigation[14] and is a common time-interval used for the classification of a delayed irrigation in retrospective studies evaluating the effect of timing of treatment on infection rates[3,4,6,8,9]. The twelve-hour interval serves as the worst-case scenario for our model (although many open fractures receive treatment well after twelve hours).

When data from basic science research are considered, our results are not surprising. Bacterial adhesion begins at around three hours, followed by aggregation and the establishment of a biofilm[15]. Previous work with *Pseudomonas aeruginosa* has shown that the development of a biofilm occurs as early as five hours after inoculation, with maturation of this biofilm by ten hours[16]. This time-course of bacterial activity helps to explain our results at the time-intervals tested.

There is not a consensus on whether the time from injury to treatment has any effect on the rate of infection, but the majority of the data from clinical studies indicates that it does not play much of a role. For example, Patzakis and Wilkins reported on 1104 open fractures and found that the timing of treatment (less than or more than twelve hours after the injury) had no effect on the infection rate[7]. Similar results were reported for the treatment of open fractures before or after six hours[3,4,6,8,9] and eight hours[5]. Kindsfater and Jonassen, however, compared the treatment of forty-seven grade-II and III open tibial fractures before or after five hours and found a significantly higher infection rate in association with fractures that were treated late[10]. One military study demonstrated a higher infection rate among soldiers who received initial irrigation of a fracture after transportation to the United States as compared with those who were managed locally after the invasion of Panama, but the actual timing of treatment in these groups was not mentioned[11]. All of those clinical studies were retrospective in nature. The possibility of selection bias is great with more severely injured patients being triaged and managed more emergently, resulting in more potential for infections in the early treatment groups. It is understandable that the performance of a prospective, randomized trial would have serious ethical and logistical concerns, and prospective longitudinal studies might be the best way to obtain valuable clinical data[17].

This animal model of a contaminated musculoskeletal injury involved the use of a genetically modified bacterium that may not be directly applicable to common clinical situations. Although limited, our results demonstrate that earlier irrigation removes more bacteria from a complex wound than does later irrigation. All of the factors that must interplay to convert a contaminated wound into an infected one are not known; however, it is likely that bacterial count is among the most important of these factors. Prospective clinical studies are needed to better define the ideal window of time needed for optimal irrigation and débridement. ∎

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